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Relationship of serum bilirubin concentrations to kidney function and albuminuria in the United States adult population. Findings from the National Health and Nutrition Examination Survey 2001–2006

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Abstract

Background: The association of serum bilirubin concentrations with kidney function and albuminuria has not been established in the US general population.
Methods: We performed a cross-sectional analysis of data from the National Health and Nutrition Examination Survey (NHANES) 2001–2006 and examined the associations of serum total bilirubin concentrations with estimated glomerular filtration rate (eGFR) and albuminuria in a nationally representative sample of 13,184 adults aged 20 years or older. eGFR was estimated using the abbreviated Modification of Diet in Renal Disease equation. Urinary albumin excretion was measured using the albumin/creatinine ratio.
Results: An eGFR <60 mL/min/1.73 m^2 and urinary albumin/creatinine ratio ≥ 30 mg/g were present in 8.1% (n = 1072) and 10.6% (n = 1402) of participants, respectively. A total of 427 (3.2%) individuals had a serum total bilirubin concentration >22.23 μmol/L. After adjustment for demographics, comorbidities, alcohol consumption, viral hepatitis status and other laboratory measures, serum bilirubin was negatively associated with eGFR, and positively with albuminuria both in the whole cohort and in the subgroup of non-diabetic individuals. In contrast, serum bilirubin was not significantly associated with eGFR or albuminuria in persons with diabetes (n = 1253).
Conclusions: Increasing serum concentrations of total bilirubin are independently associated with decreasing eGFR and increasing albuminuria in the US adult population.
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Keywords: albuminuria; bilirubin; epidemiology; estimated glomerular filtration rate; liver dysfunction.

Introduction

The prevalence of chronic kidney disease (CKD) is increasing worldwide (1, 2). Reduced estimated glomerular filtration rate (eGFR) and abnormal albuminuria have been associated with increased risk of end-stage renal disease, cardiovascular disease and other comorbidities (3–6). Because kidney disease often progresses to end-stage renal disease, the identification of precursors and risk factors for kidney disease progression are essential, with the belief that interventions will prevent or delay progression to kidney failure.

Increased concentrations of serum bilirubin have long been used as a marker of liver dysfunction. In addition, serum bilirubin has been shown to be inversely related to cardiovascular disease, suggesting a potentially athero-protective effect of bilirubin (7–10). However, the relationship of serum bilirubin with the future risk of cardiovascular events appears to be more complex. A U-shaped relationship was observed between serum bilirubin and risk of major ischemic heart disease events in a prospective study of 7685 middle-aged British men who were followed for 11.5 years (11). It is also known that serum bilirubin concentrations are significantly higher in men than in women, and tend to decline with advancing age in both sexes (12).

Information on the association of serum bilirubin concentrations with kidney function and albuminuria is limited and controversial. Fukui et al. found that serum total bilirubin was positively associated with eGFR, and negatively with albuminuria in a hospital-based sample of 633 Japanese type 2 diabetic patients (13). These results suggest a potential reno-protective effect of bilirubin. In contrast, we found that higher serum total bilirubin was significantly associated with lower eGFR in both non-diabetic and diabetic individuals in 2678 unselected outpatients 35 years or age or older (14).

To date, the association of serum bilirubin concentrations with kidney function and albuminuria have

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not been established in the US general population. We, therefore, examined the association of serum bilirubin concentrations with kidney function measures in the National Health and Nutrition Examination Survey (NHANES) 2001–2006, a nationally representative cross-sectional examination of the US adult civilian population.

Materials and methods

Study population

The NHANES is a program designed to assess the health and nutritional status of the US civilian non-institutionalized population. The study was approved by the National Center for Health Statistics (NCHS) Institutional Review Board and all subjects gave informed consent (15). The NHANES examinations were conducted from 2001 to 2006 in three phases (2001–2002, 2003–2004 and 2005–2006), and data from these phases were combined for the purpose of this analysis, following NCHS analytical guidelines (16).

In all phases of NHANES, a stratified, multistage sampling design was used, with over-sampling of non-Hispanic blacks, Mexican-Americans and persons over the age of 60 years. Individuals participated in an interview conducted at home, as well as an extensive physical examination performed at a mobile examination center, which included blood and urine collection (17). This analysis was initially restricted to 13,213 adults 20 years of age or older. However, responders who had a missing serum bilirubin and incomplete information on the main covariates of interest were excluded from analysis (n = 29). Thus, 13,184 adults constituted the sample used in this analysis.

Study variables

After collection of fasting, venous blood samples were immediately centrifuged. Specimens were then frozen and shipped weekly to a central laboratory where they were initially stored at –20°C, and then at –70°C (16, 17). Serum total bilirubin was determined by automated biochemical profiling (Beckman Synchron LX20, Beckman Coulter, Fullerton, CA, USA); fractionation of bilirubin was not performed. The LX20 uses a timed-endpoint Diazo method to measure the concentration of serum total bilirubin. The analytical range for this assay is 1.71–513 μmol/L (0.1–30 mg/dL), and the reference range is 3.42–22.23 μmol/L (0.2–1.3 mg/dL). Both intra- and inter-assay coefficients of variation were <3% (16).

eGFR was derived from the re-expressed MDRD equation = $175.0 \times (\text{serum creatinine value})^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female) × 1.21 (if black) (18). Values that exceeded 200 mL/min/1.73 m^2 were truncated. Serum creatinine was measured using the Jaffé method (kinetic alkaline picrate) with a Beckman Synchron LX20 (16, 19). As reported in the NHANES laboratory procedure manual, hemolysis, lipemia and bilirubin <34.2 μmol/L (<2 mg/dL) have no significant interference with the measurement of serum creatinine. At bilirubin levels >51.3 μmol/L (>3 mg/dL), the creatinine value is decreased by 6.84 μmol/L (0.4 mg/dL). Other potential interfering substances (e.g., cefoxitin, cefaclor, gluthathione, acetoacetic acid, L-DOPA methyl ester) are reported in detail in the laboratory procedures manual (16, 19). Both intra- and inter-assay coefficients of variation were <3.5%. As recommended by NHANES analytic guidelines (19), NHANES serum creatinine values in 2005–2006 were adjusted to ensure comparability with standard creatinine using the following formula: standard creatinine (mg/dL) = –0.016 + 0.978 × (NHANES 2005–2006 uncalibrated serum creatinine [mg/dL]). However, a recent calibration study in which the original serum creatinine measurements (Jaffé method) in NHANES 2001–2002, and 2003–2004 were compared with standard creatinine measured using an assay traceable to known gold-standard methods implemented at the Cleveland Clinic Research Laboratory demonstrated that no correction was needed for serum creatinine concentrations measured in NHANES 2001–2004 (20). In particular, the Cleveland Clinic Research laboratory analyzed the serum creatinine specimens using a Roche coupled enzymatic assay (creatininase, creatinase, sarcosine oxidase, kits #1775677 and #1775766) performed on a Roche P Module instrument (Roche Diagnostics, Basel, Switzerland). The Roche method calibrators were traceable to an isotope dilution mass spectrometric method for serum creatinine using standard references methods (NIST-SRM 967). They were also confirmed by analysis of CAP LN-24 linearity set based on National Institute of Standards and Technology (NIST) assigned values (20).

Urinary albumin excretion was measured using a fluorescent immunoassay (Sequoia-Turner model 450 digital fluorometer, Block Scientific, Holbrook, NY, USA) on the basis of the spot urine albumin/creatinine ratio. Both intra- and inter-assay coefficients of variation were <9% (16). Abnormal albuminuria was defined as albumin/creatinine ratio ≥30 mg/g (18).

Serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) were measured enzymatically with a Hitachi-704 Analyzer (Roche Diagnostics, Indianapolis, IN, USA) (16). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald's equation, except when triglycerides exceeded 4.55 mmol/L. Exposure to hepatitis C virus (HCV) was determined by presence of antibody to HCV, and hepatitis B infection was identified by the presence of hepatitis B core antibody (16). Serum glucose was measured using a modified hexokinase enzymatic method, and a radioimmunoassay method was used to measure serum insulin (16). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using fasting glucose and insulin measurements as follows [(fasting insulin (μU/mL) × fasting glucose (mmol/L))/22.5] (21). HOMA-IR score was available in non-diabetic individuals.

Self-reported race was defined as non-Hispanic white, non-Hispanic black, Mexican-American, or other. A diagnosis of hypertension was assigned if the subject reported a physician diagnosis of hypertension, if the subject reported taking prescription medications for hypertension, or if the systolic blood pressure was ≥140 mm Hg or the diastolic blood pressure was ≥90 mm Hg. Participants were defined as having diabetes if they were taking hypoglycemic drugs, had a fasting plasma glucose concentration ≥7 mmol/L or if a physician told them that they had diabetes. Smoking status was classified as never smoked, ex-smoker or current smoker. Alcohol consumption was recorded as number of drinks per day. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured with a steel measuring tape to the nearest 0.1 cm at the high point of the iliac crest at minimal respiration (16).

Statistical analysis

NHANES uses a complex, multistage, probability-sampling design to select participants that are representative of the

civilian, non-institutionalized US population. All analyses accounted for the complex sampling method. Analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NY, USA) callable SUDAAN version 9.01 (Research Triangle Institute, Research Triangle Park, NC, USA).

Results are expressed as mean ± SD or percentages. For statistical purposes (Tables 1 and 2), participants were stratified into categories of eGFR (i.e., <60, 60–89 and ≥90 mL/min/1.73 m^2) and albuminuria (classified as normal and abnormal, i.e., albumin/creatinine ratio ≥30 mg/g). In these analyses, we combined subjects with eGFR 15–30 mL/min/1.73 m^2 and those with eGFR 30–59 mL/min/1.73 m^2 into a single eGFR category due to the low number of individuals (n = 66) with eGFR values of 15–30 mL/min/1.73 m^2.

Statistical analyses included one-way analysis of variance (for continuous variables) and the χ^2-test with Yates's correction for continuity (for categorical measures). Skewed variables (triglycerides and HOMA-IR score) were logarithmically transformed to improve normality prior to analysis.

The association between serum bilirubin and eGFR (included as continuous variable) was investigated using linear (unadjusted and fully adjusted) regression models in the whole group and in subgroups of participants stratified by diabetes status (Table 3). We excluded subjects who had a serum creatinine concentration >176.8 μmol/L (>2.0 mg/dL) or serum bilirubin concentrations >22.23 μmol/L (>1.3 mg/dL) (n = 513). The covariates included in fully-adjusted regression models were age, gender, race/ethnicity, smoking status, hypertension, diabetes, BMI, waist circumference, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, alcohol consumption, viral hepatitis markers, glycosylated hemoglobin, HOMA-IR score and albuminuria.

The association between serum bilirubin and albuminuria (included as categorical variable) was investigated using logistic regression models in the whole group, excluding subjects who had a serum creatinine >176.8 μmol/L (>2.0 mg/dL) or serum bilirubin >22.23 μmol/L (>1.3 mg/dL) (n = 513), and in subgroups of participants stratified by diabetes status (Table 4). The covariates included in fully-adjusted regression models were age, gender, race/ethnicity, smoking status, hypertension, diabetes, BMI, waist circumference, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, alcohol consumption, viral hepatitis markers, glycosylated hemoglobin, insulin resistance and eGFR.

In all regression models, observations were weighted to reflect the general US population as of the early 2000s, using

Table 1 Age-gender standardized baseline demographics, comorbidities and laboratory results according to eGFR in the entire cohort (n = 13,184).

eGFR Characteristics	≥90 mL/min/1.73 m^2 n = 6987	60–89 mL/min/1.73 m^2 n = 5125	<60 mL/min/1.73 m^2 n = 1072	p-Value for trend
Age, years	38 (14)	52 (16)	71 (10)	<0.0001
Gender, %				0.01
Males	47.4	48.9	36.4	
Females	52.6	51.1	63.6	
Ethnicity, %				<0.0001
Non-Hispanic white	39.3	65.1	71.2	
Non-Hispanic black	24.7	15.0	14.0	
Mexican-American	31.5	16.5	11.3	
Other	4.5	3.4	3.5	
Smoking status, %				<0.0001
Never	53.4	51.1	50.9	
Prior	18.8	27.9	38.5	
Current	27.8	21.0	10.6	
Hypertension, %	19.5	34.5	68.1	<0.0001
Diabetes, %	6.5	8.4	23.7	<0.0001
Alcohol drinks per day, %				<0.0001
0	36.1	36	57.6	
1–3	44.8	51.9	39.7	
>3	19.1	12.1	2.7	
Measurements				
BMI, kg/m^2	28 (7)	29 (6)	29 (5)	0.001
Waist circumference, inches	38 (7)	39 (6)	40 (5)	<0.0001
Glucose, mmol/L	5.28 (1.8)	5.44 (1.8)	6.11 (2.0)	<0.0001
Total cholesterol, mmol/L	5.12 (1.1)	5.26 (1.1)	5.25 (1.1)	<0.0001
LDL cholesterol, mmol/L	2.96 (0.9)	3.01 (0.9)	2.98 (0.8)	0.0005
HDL cholesterol, mmol/L	1.38 (0.4)	1.38 (0.4)	1.37 (0.4)	0.27
Triglycerides, mmol/L	1.66 (1.8)	1.70 (1.4)	1.93 (1.0)	0.0003
AST, U/L	25 (23)	25 (13)	26 (11)	0.60
ALT, U/L	26 (26)	25 (15)	22 (11)	0.001
GGT, U/L	29 (55)	30 (34)	30 (47)	0.17
Total bilirubin, μmol/L	12.3 (5.4)	12.8 (4.6)	13.0 (6.8)	<0.0001
Glycosylated hemoglobin, %	5.5 (1.0)	5.6 (0.9)	5.9 (0.9)	<0.0001
HOMA-IR score	3.3 (3.4)	3.5 (3.9)	4.8 (5.2)	<0.0001
Albuminuria, %	8.1	10.0	30.1	<0.0001
Hepatitis B antibody, %	20.5	16.1	11.5	<0.0001
Hepatitis C antibody, %	1.0	0.9	0.5	0.20

Results are expressed as means (±SD) or percentages. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate.

Table 2 Age-gender standardized baseline demographics, comorbidities and laboratory results according to urinary albumin excretion in the entire cohort (n = 13,184).

Urine albumin/creatinine ratio, mg/g Characteristics	<30 n = 11,652	≥30 n = 1532	p-Value for difference
Age, years	44 (17)	54 (17)	<0.0001
Gender, %			0.002
Males	47.8	43.3	
Females	52.2	56.7	
Ethnicity, %			<0.0001
Non-Hispanic white	51.3	42.8	
Non-Hispanic black	20.0	25.3	
Mexican-American	24.7	27.3	
Other	4.0	4.6	
Smoking status, %			0.14
Never	52.8	49.3	
Prior	22.9	26.4	
Current	24.3	24.3	
Hypertension, %	25.2	50.6	<0.0001
Diabetes, %	6.0	26.6	<0.0001
Alcohol drinks per day, %			<0.0001
0	35.8	47.0	
1–3	48.2	39.9	
>3	16.0	13.1	
eGFR, %, mL/min/1.73 m^2			<0.0001
≥90	59.1	46.2	
60–89	36.7	36.0	
<60	4.2	17.8	
Measurements			
BMI, kg/m^2	28 (6)	30 (8)	<0.0001
Waist circumference, inches	38 (6)	40 (6)	<0.0001
Glucose, mmol/L	5.22 (1.5)	6.61 (3.2)	<0.0001
Total cholesterol, mmol/L	5.18 (1.1)	5.26 (1.2)	0.03
LDL cholesterol, mmol/L	2.97 (0.9)	3.02 (0.9)	0.02
HDL cholesterol, mmol/L	1.38 (0.4)	1.36 (0.4)	0.13
Triglycerides, mmol/L	1.65 (1.5)	2.04 (2.3)	<0.0001
AST, U/L	25 (19)	26 (14)	0.24
ALT, U/L	26 (22)	25 (15)	0.05
GGT, U/L	29 (39)	40 (81)	<0.0001
Total bilirubin, μmol/L	12.4 (4.9)	12.3 (6.3)	0.40
Glycosylated hemoglobin, %	5.5 (0.8)	6.3 (1.7)	<0.0001
HOMA-IR score	3.3 (3.4)	5.1 (5.8)	<0.0001
Hepatitis B antibody, %	18.5	16.4	0.06
Hepatitis C antibody, %	0.9	1.5	0.02

Results are expressed as means (±SD) or percentages. eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.

weights calculated for that purpose by the National Health Statistics (17). p-Values <0.05 were considered significant.

Results

Among the 13,184 adult participants, the mean serum total bilirubin was 12.48 (±5.3) μmol/L, the median was 12.0 (inter-quartile range 10.26–13.68) μmol/L. A total of 427 (3.2%) persons had a serum bilirubin concentration >22.23 μmol/L (>1.3 mg/dL). An eGFR <60 mL/min/1.73 m^2 or albumin/creatinine ratio ≥30 mg/g were present in 8.1% (n = 1072) and 10.6% (n = 1402) of the whole sample, respectively.

The clinical and biochemical characteristics of participants stratified by eGFR categories are summarized in Table 1. Compared with those with normal or near-normal eGFR, persons with lower eGFR were older, more likely to be female, more likely to be non-Hispanic white, and had greater prevalence of hypertension, diabetes and abnormal albuminuria, and higher values of BMI, waist circumference, serum glucose, glycosylated hemoglobin, insulin resistance, total cholesterol, LDL-cholesterol, triglycerides, and total bilirubin concentrations. Moreover, participants with lower eGFR also had lower serum ALT activity and were less likely to report alcohol consumption and a lower prevalence of hepatitis B seropositivity.

When participants were stratified by categories of albuminuria (Table 2), the results remained essentially unchanged with a single important exception, i.e., no significant differences were found in serum bilirubin concentrations between participants with normal and those with abnormal albuminuria. Moreover,

Table 3 Association of serum bilirubin concentrations with eGFR in NHANES participants.

	β (95% CI)	Standardized β	t-Value	p-Value
Whole cohort (n = 13,184)				
Unadjusted	−8.35 (−9.79 to −6.91)	−0.10	−11.4	<0.0001
Multivariate adjusted[a]	−3.40 (−4.71 to −2.09)	−0.04	−5.12	<0.0001
Excluding participants with a serum creatinine >2.0 mg/dL or bilirubin >1.3 mg/dL (n = 12,671)				
Unadjusted	−16.22 (−18.30 to −14.14)	−0.14	−15.3	<0.0001
Multivariate adjusted[a]	−8.41 (−10.35 to −6.47)	−0.07	−8.51	<0.0001
Non-diabetics (n = 11,931)				
Unadjusted	−9.35 (−10.82 to −7.88)	−0.11	−12.50	<0.0001
Multivariate adjusted[b]	−4.12 (−5.45 to −2.79)	−0.05	−6.09	<0.0001
Diabetics (n = 1253)				
Unadjusted	1.61 (−4.88 to 8.10)	0.01	0.5	0.63
Multivariate adjusted[c]	2.06 (−5.07 to 9.19)	0.01	0.6	0.57

[a]Adjusted for age, gender, race/ethnicity, smoking, hypertension, diabetes, body mass index, waist circumference, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, alcohol consumption, viral hepatitis markers, glycosylated hemoglobin, albuminuria, and homeostasis model assessment of insulin resistance. [b]Adjusted for age, gender, race/ethnicity, smoking, hypertension, diabetes, body mass index, waist circumference, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, alcohol consumption, viral hepatitis markers, homeostasis model assessment of insulin resistance, and albuminuria. [c]Adjusted for age, gender, race/ethnicity, smoking, hypertension, diabetes, body mass index, waist circumference, glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, alcohol consumption, viral hepatitis markers, glycosylated hemoglobin and albuminuria. CI, confidence interval; eGFR, estimated glomerular filtration rate; NHANES, National Health and Nutrition Examination Survey.

Table 4 Association of serum bilirubin concentrations with urinary albumin excretion ≥30 mg/g in NHANES participants.

	Odds ratio (95% CI)	p-Value
Whole cohort (n = 13,184)		
Unadjusted	0.92 (0.75–1.12)	0.40
Multivariate adjusted[a]	1.26 (1.03–1.54)	0.02
Excluding participants with a serum creatinine >2.0 mg/dL or bilirubin >1.3 mg/dL (n = 12,671)		
Unadjusted	0.88 (0.67–1.15)	0.35
Multivariate adjusted[a]	1.32 (0.95–1.85)	0.09
Non-diabetics (n = 11,931)		
Unadjusted	1.05 (0.85–1.29)	0.68
Multivariate adjusted[b]	1.24 (1.01–1.54)	0.04
Diabetics (n = 1253)		
Unadjusted	1.04 (0.63–1.73)	0.88
Multivariate adjusted[c]	1.37 (0.76–2.48)	0.30

[a]Adjusted for age, gender, race/ethnicity, smoking, hypertension, diabetes, body mass index, waist circumference, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, alcohol consumption, viral hepatitis markers, glycosylated hemoglobin, homeostasis model assessment of insulin resistance, and estimated glomerular filtration rate. [b]Adjusted for age, gender, race/ethnicity, smoking, hypertension, diabetes, body mass index, waist circumference, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, alcohol consumption, viral hepatitis markers, homeostasis model assessment of insulin resistance, and estimated glomerular filtration rate. [c]Adjusted for age, gender, race/ethnicity, smoking, hypertension, diabetes, body mass index, waist circumference, glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein-cholesterol, triglycerides, alcohol consumption, viral hepatitis markers, glycosylated hemoglobin, and estimated glomerular filtration rate. CI, confidence interval; NHANES, National Health and Nutrition Examination Survey.

persons with abnormal albuminuria also had higher serum GGT activity and higher prevalence of hepatitis C seropositivity.

When the analyses described above were limited to persons diagnosed with diabetes (n = 1253), serum total bilirubin concentrations did not change across categories of eGFR (11.8 ± 4.4 vs. 11.3 ± 3.6 vs. 12.0 ± 3.6 μmol/L from the 1st–3rd eGFR category, respectively; p = 0.95 for trend) and albuminuria (11.6 ± 3.9 vs. 11.8 ± 3.8 μmol/L normal vs. abnormal albuminuria; p = 0.87).

When we performed a subgroup analysis of participants stratified by age groups (Figure 1), serum bilirubin concentrations significantly increased across eGFR categories, i.e., with higher serum bilirubin in participants with lower eGFR (p < 0.05 or less for each age group). In contrast, serum bilirubin concentrations did not significantly change across albuminuria categories stratified by age groups (data not shown).

Table 3 shows the association between serum total bilirubin and eGFR (included as continuous variable) in univariate and multivariate linear regression mod-

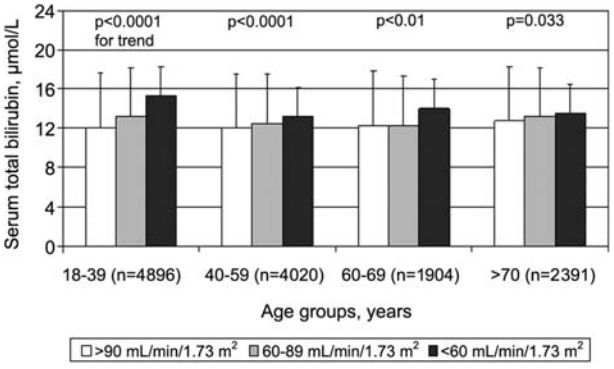

Figure 1 Mean (±SD) serum bilirubin concentrations by age and estimated glomerular filtration rate (eGFR) categories in the NHANES participants (n = 13,184).
Differences for trends in serum bilirubin for each age group were tested by one-way analysis of variance.

els. In univariate analysis, total bilirubin was inversely associated with eGFR in the whole sample, in the non-diabetic subgroup, and after excluding subjects with a serum creatinine >176.8 μmol/L or a total bilirubin concentration >22.23 μmol/L. No significant association was found between serum bilirubin and eGFR in the diabetic subgroup. These results remained essentially unchanged after adjustment for demographics, comorbidities, alcohol consumption, viral hepatitis status, albuminuria and other laboratory measures.

Table 4 shows the association between serum total bilirubin and albuminuria – included as categorical measure – in unadjusted and fully adjusted logistic regression models. Serum bilirubin did not correlate with abnormal albuminuria in unadjusted logistic regression models, neither in the whole cohort nor in other subgroups of participants. Conversely, the association between serum bilirubin and abnormal albuminuria became significant after adjustment for demographics, comorbidities, alcohol consumption, viral hepatitis status, eGFR and other laboratory measures, both in the whole cohort and in non-diabetics, but not in diabetic persons.

Discussion

To our knowledge, this is the first population-based study specifically aimed at examining the association between serum bilirubin concentrations and kidney function measures in the general population. In a nationally representative sample of US adults, we found a significant, inverse association between normal serum total bilirubin concentrations and eGFR. This association was independent of a broad spectrum of established risk factors and potential confounders, such as age, gender, race/ethnicity, smoking status, hypertension, diabetes, BMI, waist circumference, plasma lipids, glucose, insulin resistance, viral hepatitis status, alcohol consumption, and albuminuria. Notably, the inverse association between serum total bilirubin and eGFR was significant in non-diabetic individuals and after excluding participants with laboratory evidence of advanced kidney dysfunction (defined as a serum creatinine concentration >176.8 μmol/L) or possible liver disease (defined as a serum bilirubin concentration >22.23 μmol/L). Similarly, we found a positive, but much weaker, association between serum total bilirubin and abnormal albuminuria in the whole cohort and in the non-diabetic subgroup. In contrast, we did not find any association between serum bilirubin with eGFR and albuminuria in diabetic persons.

These findings partly confirm and extend our recent observations in a large hospital-based sample of 2678 adult outpatients (mean age: 55±18 years; 43% male), 210 with diabetes (14). In that study, we found that serum total bilirubin was inversely associated with eGFR in both non-diabetic ($r = -0.17$; $p < 0.0001$) and diabetic patients ($r = -0.14$; $p < 0.05$). However, no information was available on albuminuria, comorbidities, alcohol consumption and other important potential confounders (14).

The lack of a significant association between serum bilirubin and eGFR or albuminuria observed in diabetic individuals of our cohort is in contrast with the results by Fukui et al. (13). These authors found that serum total bilirubin was positively associated with eGFR, and negatively with albuminuria in a hospital-based sample of 633 Japanese type 2 diabetic patients (mean age: 64.4±11.5 years; 52% male). In that study, the inverse association between serum bilirubin and albuminuria (standardized β-coefficient = –0.19; $p < 0.0001$) was independent of age, gender, duration of diabetes, BMI, smoking status, blood pressure, plasma lipids and glycosylated hemoglobin. However, in that study, no adjustment was made for important confounders, such as eGFR, alcohol consumption and viral hepatitis status (13).

We believe that the apparently discrepant results regarding an association, if any, between serum bilirubin and kidney function measures in the diabetic population (13, 14) can be largely explained by the larger sample size of the present study and, principally, by differences in the study population (for example, different distribution of age, gender, ethnicity and comorbidities) and study methodology (general population vs. hospital-based cohort).

Clearly, we must be cautious in making any causal inference, given the cross-sectional design of our study. The exact mechanism(s) linking higher serum bilirubin and decreased kidney function measures are not fully understood. The most obvious explanation for our findings is that the greater prevalence of lower eGFR in persons with higher serum bilirubin concentrations simply reflects the coexistence of established risk factors for kidney damage. However, since in our study serum bilirubin was associated with decreasing eGFR and rising albuminuria independently of several established risk factors, it is conceivable that serum bilirubin might confer an excess risk over and above the risk expected as a result of the established and more traditional risk factors. In clinical practice, an increased serum bilirubin concentration is conventionally interpreted as a marker of liver dysfunction,

including cholestasis, drug-induced hepatitis, viral hepatitis, Gilbert's syndrome and others (22). In our study, however, serum bilirubin was significantly associated with decreasing eGFR after excluding subjects with a serum bilirubin concentration >22.23 μmol/L, and even after adjusting for alcohol intake and viral hepatitis status. Thus, alcohol consumption, viral hepatitis and other advanced liver diseases are unlikely to fully explain the association of serum bilirubin concentrations with worsening kidney function. In addition, as reported previously, the exclusion of persons with a serum bilirubin concentration >22.23 μmol/L minimizes any significant laboratory interference by bilirubin with the measurement of serum creatinine.

It could be hypothesized that the bilirubin-kidney dysfunction association primarily reflects the association of serum bilirubin concentrations with non-alcoholic fatty liver disease (NAFLD). NAFLD is now regarded as the hepatic manifestation of the metabolic syndrome and represents the most common cause of mild to moderate increases in serum bilirubin and other liver enzymes in Western countries (23–25). NAFLD can promote atherogenic dyslipidemia and contribute to CKD pathogenesis – as well as to accelerated atherogenesis – through the release of some pathogenetic mediators from the steatotic liver, including increased C-reactive protein and other inflammatory cytokines (26). Importantly, several studies have shown that these potential mediators of vascular and kidney damage are markedly higher in patients with NAFLD than in those without (26–31), and are thought to be pathogenic factors for the development of CKD (32–35). Consistent with the hypothesis that liver inflammation (or other liver-derived factors) in NAFLD may play a role in kidney disease progression, it has been shown that type 2 diabetic subjects with chronic hepatitis B virus infection were more likely to develop end-stage renal disease compared with those not infected with hepatitis B virus (36). More importantly, recent studies found that NAFLD is independently associated with an increased incidence of CKD in both non-diabetic and diabetic populations (37, 38).

Limitations of our study include its cross-sectional design, which allows us to identify associations and should not yield any conclusions about causation. Second, NHANES only measured total and not fractionated bilirubin concentrations (thus, excluding the possibility to discriminate the Gilbert's disease from other liver disease), and performed only a single blood draw for measuring serum bilirubin, perhaps limiting the precision of the bilirubin measurement. Third, liver ultrasonography for diagnosing NAFLD was not performed. Finally, we used the eGFR instead of a directly measured GFR to assess kidney function. It is known that current GFR estimates have greater inaccuracy in populations without known CKD than in those with kidney disease. Nonetheless, current GFR estimates facilitate the detection, evaluation, and management of kidney disease. Also, many organizations recommend the use of prediction equations for the evaluation of kidney function in large epidemiologic studies and in clinical practice (18).

Despite these limitations, our analysis has several important strengths. First, it is the most comprehensive national survey to examine the associations of serum total bilirubin with kidney function and albuminuria in US adults. Second, NHANES used uniform methods to collect data on demographics, comorbidities and laboratory measures. Third, the extensive and complete data on important factors associated with kidney disease and serum bilirubin increases allows us to give an unbiased estimate for the relationship between serum bilirubin and CKD. Finally, with the design of NHANES, we are able to generalize the results to the entire US civilian non-institutionalized population.

In conclusion, our findings suggest that increasing serum total bilirubin concentrations are independently associated with decreasing eGFR and increasing albuminuria in the US adult population. Future prospective studies are required to determine the temporal nature of this association.

Conflict of interest statement

We declare that we have not accepted any funding or support from an organization that may in any way gain or lose financially from the results of your study, that we have not been employed by an organization that may in any way gain or lose financially from the results of our study, and that we do not have any other conflicting interests.

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